Decoration of Amphiphilic NDI-diacetylene Nanotubes with Gold Nanoparticles and the Anti-parallel β-Sheet Assembly of Porphyrin Modified Tetrapeptides

Thesis

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Abstract

Self-assembly is crucial to numerous systems that occur in nature; researchers have often attempted to mimic these natural systems by utilizing non-covalent interactions of amphiphilic systems to form self-assembled structures in solution for useful applications. The Parquette group has discovered a self assembling 1,4,5,8-naphthalenediimide (NDI) diacetylene system with a lysine unit that assembles into a robust one-dimensional nanotube, capable of acting as a surfactant for gold nanoparticle growth. The diacetylene unit is capable of polymerization, as well as changing its conformation to sheets after heating; regardless of the shape or polymerization status of the nanostructures, they were able to act as a stabilized surface for gold nanoparticle growth. Other groups have discovered that gold nanoparticles are attracted to amine functionality electrostatically, decorating the surface of a surfactant. However, there are few examples of self-assembled systems forming nanostructures that can withstand the slightly acidic Turkevich method of reducing gold (III) into gold (0) using citric acid or citrate.

Stimuli-responsive materials have been of interest in recent years, due to the interest in mimicking various types of biological systems for different applications. Self-assembling systems are an interesting approach to this research due to its ability to
create highly ordered systems quickly. Stimuli-responsive materials react to their chemical and physical environments; therefore, by manipulation of a molecular structure, it is possible to develop a unique set of compounds that react to their environments for applications in optoelectronics, drug delivery, or bio-mimics. In this work, two tetrapeptides with the sequence Phe-Lys-Lys(TPP-Zn)-Lys were developed using a tetraphenylporphyrin ring on the third lysine tail. The difference in the peptides was that one had a free amine on the lysine adjacent to the phenylalanine amino acid, while the other peptide had an acyl-protecting group on the lysine adjacent to the phenylalanine. Due to the Zn-TPP chromophore on the assembly, nitrogen-containing ligands were used to make the structure more defined. With the addition of DABCO or pyridine, the observed structures based on microscopy methods changed depending on the conditions. The tetrapeptides formed β-sheet structures based on IR data; since anti-parallel and parallel β-sheets are possible, an isotopic equivalent of each tetrapeptide was synthesized and analyzed by IR. A splitting of the amide I band demonstrates the formation of anti-parallel β-sheets when the isotope is used; in this case, the amide I band split for both isotopic equivalents indicating anti-parallel β-sheet formation.
Dedication

This thesis is dedicated to my family for supporting me through all decisions I ever make.

I couldn’t have done it without you.
Acknowledgments

I would first and foremost like to thank the Chemistry Department at Ohio State for helping me learn so much in such a limited amount of time. I would also like to thank my advisor, Jon Parquette for being a source of knowledge and guidance during my time working with him. Also, to Nick Bewick who has been a mentor and friend in the lab and out. You always have the answers to my questions and I couldn’t be more thankful for all of your help. To Keisha Neidrich, I literally wouldn’t have survived this time without you. Also, thank you to all my lab mates and classmates who have been such a source of support. And finally to my family, I thank you for your constant love and guidance through it all.

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# Table of Contents

Abstract .......................................................................................................................... ii

Dedication ...................................................................................................................... iv

Acknowledgments ........................................................................................................ v

Vita ................................................................................................................................ vi

List of Tables .................................................................................................................. x

List of Figures ................................................................................................................ xi

List of Abbreviations ...................................................................................................... xvi

Chapter 1 : Self-Assembly ............................................................................................ 1

1.1 Introduction ............................................................................................................. 1

1.2 Non-covalent Interactions ...................................................................................... 1

1.3 Amphiphilic self-assembly ..................................................................................... 3

1.3.1 Peptide amphiphiles ......................................................................................... 4

1.3.2 Chromophores in Self-Assembly ....................................................................... 7

1.4 Applications of Amphiphilic Self-Assembly ............................................................ 9

1.4.1 Drug Delivery using Self-Assembled Nanostructures ........................................ 9
| 1.4.2 Encapsulation using Amphiphilic Self-Assembly | 10 |
| 1.5 NDI-lysine Amphiphilic Self-Assembly | 11 |
| 1.6 References | 13 |
| Chapter 2: Decoration of an Amphiphile with Gold Nanoparticles | 17 |
| 2.1 Introduction | 17 |
| 2.2 Research Design | 18 |
| 2.3 Research | 24 |
| 2.4 Results and Discussion | 26 |
| 2.5 Conclusion | 44 |
| 2.6 Methods and Experimentals | 45 |
| 2.7 References | 54 |
| Chapter 3: Controllable, stimuli-responsive assembly of anti-parallel β-sheet forming nanofibers | 57 |
| 3.1 Introduction | 57 |
| 3.2 Research Design | 59 |
| 3.2.1 Metal-ligand coordination assists assembly process | 60 |
| 3.2.2 Anti-parallel vs. Parallel β-Sheet IR studies | 61 |
| 3.3 Results and Discussion | 63 |
| 3.4 Conclusion | 78 |
List of Tables

Table 2.1 Titration of compound A (500 µM) and citric acid (500 µM) with HAuCl₄ (1 mM)......................................................................................................................... 30
List of Figures

Figure 1.1 Summary of non-covalent interactions that can be involved in self-assembly.13
                                                                                                                                                                                                                                                                     2
Figure 1.2 General four-part structure of the Stupp groups’ peptide amphiphile.18 ........... 6
Figure 1.3 Basic structure of a NDI molecule (left) and a porphyrin molecule (right). ..... 8
Figure 1.4 Two drug amphiphiles used by Lock and coworkers for drug delivery.36 ...... 10
Figure 1.5 Amphiphilic NDI lysine structure developed by Shao and coworkers.46 ........ 12
Figure 1.6 UV-vis spectrum of dipeptide A demonstrating a red shift from TFE to water.
                                                                                                                                                                                                                                                                     13
Figure 2.1 The Turkevich method of reducing gold(III) to gold(0) (A). Two ways gold can
electrostatically interact with cations; the non-reduced gold(III)tetrachloride anion or
the reduced colloidal gold coated with citrate can interact with a positively charged
surface, in this case amines (B). .................................................................................................................. 19
Figure 2.2 Carbon nanotube functionalized with amines interacting with citric acid to
attract gold nanoparticles (A). MWNT functionalized with amines (B). Functionalized
MWNT coated with gold nanoparticles (C).69 ....................................................................................... 20
Figure 2.3 The chemical structures of amphiphilic pillar[5]arene (A) and the non-cyclic
monomeric analog (B). Schematic illustration of the microtube decorated with gold
nanoparticles (C). TEM images of the microtube decorated with gold nanoparticles
(D), the vertical section (E) and the cross section (F).\textsuperscript{60} .................................................................................. 22

Figure 2.4 Structure of the hexapeptide (A). TEM image of the peptide coated with gold nanoparticles (B). Self-assembly structure of the peptide fibers with and without gold nanoparticles (C) ........................................................................................................................................ 24

Figure 2.5 Amphiphilic compound A assembled and decorated with gold nanoparticles (Picture taken from the thesis of Nicholas Bewick). .................................................................................. 26

Figure 2.6 TEM image of 1 eq. of citric acid in solution of compound A (500 µM), followed by addition of tetrachloroauric acid (0.67 eq.) aged for 1 d at pH = 3.0 (A). ........................................ 27

Figure 2.7 TEM images of compound A (500 µM) and tetrachloroauric acid (333 µM) followed by addition of citric acid (500 µM) ........................................................................................................ 28

Figure 2.8 TEM images of compound A decorated with gold nanoparticles in water (250 µM, 10 eq. HAuCl\textsubscript{4}, and 250 µM citric acid) (A and B). ................................................................. 31

Figure 2.9 TEM images using the amphiphile NH\textsubscript{2}-Lys(NDI-\textit{n}Bu)-OMe (compound B, 500 µM), sodium citrate (1 eq.), and HAuCl\textsubscript{4} (0.67 eq.) in water after 1 day of aging.... 32

Figure 2.10 TEM images of compound A (500 µM) with citrate (1 eq.) and HAuCl\textsubscript{4} (0.67 eq) aged for 1 day in water. ............................................................................................................. 34

Figure 2.11 TEM images of compound A (500 µM), with sodium citrate (500 µM) and HAuCl\textsubscript{4} (333 µM) aged for 1 day (A), 3 days (B), and 7 days (C) ......................................................... 35

Figure 2.12 UV of gold (III) reduced with sodium citrate after 0 h (red) and 24 h (blue) (A). UV of compound A dissolved with citric acid (red) and citrate (blue) in water (B). UV vis spectrum of compound B dissolved with citric acid (red) and citrate (blue) in water (C). UV vis spectrum of compound B dissolved with citrate (1 eq) and gold (III)
(0.67 eq) after 0 h (red) and 48 h (blue) (D). .......................................................... 37

Figure 2.13 TEM image of compound A (500 µM), citric acid (500 µM) and HAuCl₄ (333 µM) (A). TEM image of compound A (500 µM), citrate (500 µM) and HAuCl₄ (333 µM) (B). TEM image of compound A (500 µM), citric acid (1 mM) and HAuCl₄ (333 µM) (C). TEM image of compound A (500 µM), citrate (1 mM) and HAuCl₄ (333 µM) (D).......................................................... 39

Figure 2.14 UV spectrum at various time points during the polymerization process of compound A in water (254 nm (19 W); aged at 5 mM then diluted to 300 µM) irradiated over 30 min (A); compound A heated to 130 °C for 30 min in water in a pressure tube at 300 µM, irradiated over 30 min (B).......................................................... 41

Figure 2.15 TEM image after induced polymerization of compound A (500 µM), using UV light (254 nm, 19 W), with citric acid (500 µM), and HAuCl₄ (333 µM) (A); TEM image after induced polymerization of compound A (500 µM), using UV light (254 nm, 19 W), with citrate (500 µM), and HAuCl₄ (333 µM) (B).......................................................... 42

Figure 2.16 TEM image of compound A (500 µM) with citrate (500 µM) and tetrachloroauric acid (333 µM) in water after 12 h (A); TEM image of compound A (500 µM) with citrate (500 µM) and tetrachloroauric acid (333 µM) in water after 5 days (B); TEM image of compound A (500 µM) with citrate (500 µM) and tetrachloroauric acid (333 µM) in water after 6 days (C). .......................................................... 43

Figure 2.17 TEM images of polymerized sheets of compound A by heating compound A (2 mM) to 130 °C for 30 minutes, then shining UV-light (254 nm, 19 W) on the resulting solution for 30 minutes; TEM image of compound A (500 µM), citrate (500 µM), and
HAuCl$_4$ (333 µM) in water after 12 h (A); TEM image of compound A (500 µM), citric acid (500µM), and HAuCl$_4$ (333 µM) in water after 12 h (B).

Figure 3.1 Tetrapeptides used in β-sheet formation; Fmoc-FK(ac)K(ZnTPP)K-NH$_2$ (A) and Fmoc-FKK(ZnTPP)K-NH$_2$ (B).

Figure 3.2 Exciton band structures and IR spectra of anti-parallel and parallel β-sheets with their differences highlighted in the red box.$^{108}$

Figure 3.3. UV-vis spectra of compound 3.1 (250 µM) in CHCl$_3$ with no ligand (red), DABCO (1 eq, blue), or pyridine (2 eq, green) (A); UV-vis spectra of compound 3.2 (250 µM) with no ligand (red), DABCO (1 eq, blue) or pyridine (2 eq, green) (B).

Figure 3.4 UV-vis spectra for titrations using compound 3.1 with DABCO (A); UV-vis spectra for titrations using compound 3.1 and pyridine (B); UV-vis spectra for titrations using compound 3.2 with DABCO (C); UV-vis spectra for titrations with pyridine (D).

Figure 3.5 CD spectra for compound 3.1 with no ligand (red), DABCO (1 eq, blue), or pyridine (1 eq, green) (A); CD spectra for compound 3.2 with no ligand (red), DABCO (1 eq, blue), or pyridine (1 eq, green) (B).

Figure 3.6 TEM image of compound 3.1 in chloroform with no ligand (A), with DABCO (B), or with pyridine (C); TEM image of compound 3.2 in chloroform with no ligand (D), with DABCO (E), or with pyridine (F).

Figure 3.7 Hydrogen bonding difference between parallel and anti-parallel β-sheets.

Figure 3.8 IR spectra for compound 3.1 in chloroform with no ligand (black), with DABCO (red), and with pyridine (blue) (A). IR spectra for compound 3.3 in chloroform
with no ligand (black), with DABCO (red), and with pyridine (blue) (B). IR spectra for
### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>1D</td>
<td>one dimensional</td>
</tr>
<tr>
<td>2D</td>
<td>two dimensional</td>
</tr>
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<td>$\alpha$</td>
<td>alpha</td>
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<tr>
<td>Ac</td>
<td>Acetyl</td>
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<td>AFM</td>
<td>atomic force microscopy</td>
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<td>B</td>
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<td>circular dichroism</td>
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<td>chloroform</td>
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<tr>
<td>CH$_3$CN</td>
<td>acetonitrile</td>
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<tr>
<td>$\delta$</td>
<td>chemical shift in NMR</td>
</tr>
<tr>
<td>d</td>
<td>doublet (spectra); day(s)</td>
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<tr>
<td>DABCO</td>
<td>1,4-diazabicyclo[2.2.2]octane</td>
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<tr>
<td>DCM</td>
<td>dichloromethane</td>
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<tr>
<td>DMAP</td>
<td>4-(N,N-dimethylamino)pyridine</td>
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<tr>
<td>DMF</td>
<td>N,N-dimethylformamide</td>
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Eq. equivalent
ESI electrospray ionization
Fmoc fluorenylmethyloxycarbonyl
FTIR fourier transform infrared spectroscopy
g gram(s)
H or \(^1\)H proton
h hour(s)
HAuCl\(_4\) tetrachloroauric acid
HPLC High performance liquid chromatography
IR infrared
\(J\) coupling constant in Hz (NMR)
K\(_2\)CO\(_3\) potassium carbonate
KOH potassium hydroxide
Lys lysine
m milli; meter(s); multiplet (NMR)
\(\mu\) micro
M moles per liter
\(M\) left handed helix
Me methyl
MeOH methanol
MgSO\(_4\) magnesium sulfate
MHz megahertz
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<td>NaBH₄</td>
<td>sodium borohydride</td>
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<td>NMR</td>
<td>nuclear magnetic resonance</td>
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<td>p</td>
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<tr>
<td>P</td>
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<tr>
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<td>phenylalanine</td>
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<td>$-\log[H^+]$</td>
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<td>parts per million</td>
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<tr>
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<td>quartet</td>
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<tr>
<td>rt</td>
<td>room temperature</td>
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<td>singlet (NMR); second(s)</td>
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<tr>
<td>t</td>
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<td>t or tert</td>
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<tr>
<td>TEM</td>
<td>transmission electron microscopy</td>
</tr>
<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
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<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
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<tr>
<td>TLC</td>
<td>thin layer chromatography</td>
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<td>Description</td>
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<tr>
<td>TPP</td>
<td>tetraphenyl porphyrin</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
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<td>Zn</td>
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Chapter 1: Self-Assembly

1.1 Introduction

The process of a building block organizing into a pattern or structure without any probing or direction from an outside source is defined as self-assembly. There are numerous examples of self-assembling materials already known in nature including compounds like nucleic acids, surfactants, viruses, lipids, block co-polymers, dendrimers, and peptides. Often, researchers have strived to mimic the behaviors of naturally-occurring materials to use for various applications including drug delivery, optoelectronics, and encapsulation. The molecular interactions of self-assembling structures can be difficult to predict, due to the fact that non-covalent interactions are often the driving force for self-assembly. The types of non-covalent interactions involved often include hydrogen bonding, van der Waals interactions, electrostatic, and hydrophobic interactions, including $\pi$-$\pi$ stacking. Non-covalent interactions (2-250 kJ/mol) are much weaker compared to covalent bonds (100-400 kJ/mol), however, if there are a sufficient number of the non-covalent forces in a system, the system can be highly stable.

1.2 Non-covalent Interactions

Non-covalent forces are often the main contributor to self-assembling systems, with the most critical being $\pi$-$\pi$ stacking and hydrogen bonding. A summary of these non-covalent forces is shown in figure 1.1. The stacking of $\pi$-systems is defined as an
attractive interaction between aromatic groups, contributing to intramolecular stability.$^{13}$ The simplest example of this is the stacking of two benzene rings, with their most energetically favorable stacking arrangement being parallel displacement, and T-shaped edge-to-face (figure 1.1).$^{13}$

![Figure 1.1 Summary of non-covalent interactions that can be involved in self-assembly.](image)

In water, the source of $\pi-\pi$ stacking interactions is a result of the hydrophobic effect. When water molecules solvate an aromatic surface, the aromatic surfaces begin to stack, reducing the total surface exposed to the solvent.$^{14}$ If the solvent is not as polar,
these non-covalent forces are weaker, thus playing a more minor role in the self-assembly.

Hydrogen bonding has also proved to be critical in the self-assembly of monomers into organized structures. The most common example of hydrogen bonding in nature is base pairing in DNA. The complementary base pairs include adenine and thymine, adenine and uracil, and guanine and cytosine. Hydrogen bonds involve a donor, often N-H, O-H, or S-H bonds, and an acceptor, which is normally a heteroatom with a lone pair of electrons including N, O, S, or F. This type of non-covalent interaction is highly selective and directional, with the strength of a typical hydrogen bond depending heavily on the solvent and number of hydrogen bonds.

1.3 Amphiphilic self-assembly

An amphiphile, by definition, consist of two parts with differing characteristics, like a hydrophilic section and a hydrophobic section. Surfactants are typically characterized as the most common amphiphile, and they are distinguished as ionic or nonionic based on the charge of their hydrophilic moiety. Amphiphilicity is a strong driving force for self-assembly, due to the tendency of hydrophobic moieties to avoid contact with hydrophilic solvents like water. The aggregation of amphiphilic type molecules can minimize this unfavorable interaction, by assembling so that the hydrophilic portions are exposed to water, and the hydrophobic parts are embedded on the inside of the structure.
1.3.1 Peptide amphiphiles

Another common type of amphiphile for self-assembly is a peptide amphiphile (PA). Peptide amphiphiles contain a short peptide sequence that has overall hydrophillicity combined with a hydrophobic segment, often an alkyl chain or a sequence of hydrophobic amino acids. This type of molecule utilizes the bioactivity of a peptide sequence with the structural design of a standard amphiphile. The driving force for these types of amphiphiles comes from the aggregation of the hydrophobic segment of the amphiphile in aqueous solution. However, to get peptide amphiphiles to self-assemble into different types of structures, it seems that the hydrophilic peptide portion of the amphiphile becomes a key component in the formation of fibers as opposed to micelle type structures; this can lead to controllable assembly.

There have been numerous design strategies to control the self-assembly of peptide amphiphiles into various types of structures including nanofibers, nanovesicles, nanotubes, and nanobelts. They possess surfactant like properties causing them to assemble into well-ordered nanostructures in aqueous solution. The length of the hydrophobic tail contributes significantly to the solubility of the peptide amphiphile; if the hydrophobic tail contains amino acids, it is generally 3-9 hydrophobic amino acids, if it is an alkyl chain it is often 12-16 carbons long. By decreasing the tail length, solubility can be improved; this can also be accounted for if the size of the hydrophobic amino acid is increased. Another way to adjust the amphiphilicity is to adjust its structure to switch the hydrophobic and hydrophilic portions of the peptide chain; often the hydrophobic moiety is on the N-terminus, while the hydrophilic is on the C-terminus. If these are swapped, a difference in structure can occur. By manipulation of the non-
covalent interactions that hold self-assembled peptide amphiphiles together, the overall structure and assembly conditions adjust.\textsuperscript{18} Hartgerink \textit{et. al.} determined that the amino acids closest to the center of a nanofiber accounts for the shape of the nanostructure that is formed.\textsuperscript{22} Therefore, by adjusting these amino acids, and therefore adjusting their hydrogen bonding sites, changes the nanostructure and the assembly behavior.\textsuperscript{18} Overall, slight changes in the structure of a peptide amphiphile can completely alter the self-assembly mechanism and structure.

Velichko and coworkers also investigated the interactions that contribute to the self-assembly of peptide amphiphiles in water by using molecular simulation.\textsuperscript{23} They modeled specifically hydrophobic interactions and intermolecular hydrogen bonding between amino acids to predict the shapes of the nanostructures.\textsuperscript{23} In reference to exclusively hydrophobic interactions, the peptide amphiphiles should form micelle structures with fixed size.\textsuperscript{23} On the other hand, looking at specifically hydrogen bonding interactions, the structure that was predicted was a stepwise aggregation of the molecules into one-dimensional $\beta$-sheet structures.\textsuperscript{23} Depending on the strength of the hydrogen bonding or hydrophobic interactions, peptide amphiphiles can have a mixture of micelle and beta-sheet structures, or exclusively one or the other. For example, if the amino acids used increase the strength of the hydrogen bonding interactions, the energy of those interactions break micelle structures into long, cylindrical nanofibers where $\beta$-sheets develop along the axis.\textsuperscript{23} Thus, the combination of strong hydrogen bonding interactions and the hydrophobic interactions of the non-polar tails contributes to the development of nanofibers in aqueous solution.
Stupp and coworkers designed and synthesized several peptide amphiphiles for the self-assembly of biomaterials.\textsuperscript{22, 24,22,25,25b,26} Stupp’s group used a specific supramolecular design for their classes of peptide amphiphiles allowing for individual assembling molecules to encapsulate and deliver small, hydrophobic drugs.\textsuperscript{18} The supramolecular design Stupp \textit{et. al.} used to make peptide amphiphiles consisted of four specific structural components; a hydrophobic domain, a short peptide sequence for hydrogen bonding, a region of charged amino acids to assist with solubility, and finally a component containing a bioactive segment, shown in figure 1.2.\textsuperscript{18} Non-covalent interactions are used to drive the self-assembly of these structures into highly organized nanofibers; the major contributors are hydrogen bonding from the beta-sheet forming peptide sequence, hydrophobic interactions of the hydrophobic tail, and electrostatic repulsion of the charged amino acid section.\textsuperscript{18} The assembly of these peptide amphiphiles could then be utilized to encapsulate molecules that are hydrophobic, like semiconducting materials or drugs.\textsuperscript{18}

![Figure 1.2 General four-part structure of the Stupp groups’ peptide amphiphile.\textsuperscript{18}](image-url)
1.3.2 Chromophores in Self-Assembly

Photosynthesis is one of the most useful reactions used in nature to produce energy. Researchers have been able to use important concepts from chlorophyll’s role in transforming light into energy, during photosynthesis, to design compounds that can absorb light and be used in electron transport. Several types of chromophores are often used for electron transfer, including structures like porphyrins, phthalocyanines, perylenes, merocyanines, and naphthalene derivatives. Due to the excellent light-harvesting properties of chromophores, they have been used in self-assembled systems in aqueous solution, by combining electrostatic and hydrophobic interactions as the driving force for the assembly.

Out of the many classes of chromophores, 1, 4, 5, 8-naphthalenediimides (NDI) have been found to be particularly beneficial due to their electron deficient nature, and their ability to form n-type semiconductive materials as opposed to p-type. Naphthalene diimides contain a hydrophobic naphthalene core with two hydrophilic imide groups (figure 1.3); depending on the substitution of the imide groups, NDI molecules can be soluble in non-polar or polar solvents. Compounds made with NDI moieties have been useful in electron transfer design strategies due to the fairly straightforward synthesis of NDI molecules, and the fact that they are known electron acceptors; the electron accepting properties of an NDI molecule is crucial due to its similarities to naturally occurring acceptors often found in plants for photosynthetic uses.
Another type of chromophore that has been found to have interesting photophysical properties is a porphyrin. The simplest porphyrin is composed of four pyrrole subunits with a methine connection between each pyrrole molecule (figure 1.3); they are often substituted with other systems, adding to their already high degree of conjugation. The fact that porphyrin molecules have extensive conjugation makes them optimal candidates in self-assembly due to the $\pi-\pi$ stacking interactions; their structure has often been exploited for making new supramolecular structures. The synthesis of various types of porphyrins is well established, making the investigations into the self-assembly of porphyrin structures of interest over the past several years. Since chromophores, like porphyrin molecules, are often large and hydrophobic, self-assembly is one of the simplest ways to design a library of large chromophoric molecules with
excellent electronic and structural properties.\textsuperscript{31, 28} Porphyrin moieties can be used in dyad or triad systems as electron donors.\textsuperscript{28}

1.4 Applications of Amphiphilic Self-Assembly

Since peptide amphiphiles have the potential to form numerous types of nanostructures including micelles, nanofibers, nanotubes, or nanobelts,\textsuperscript{21} they have promising applications in drug and cell delivery, gene therapy, and optoelectronics, often through non-covalent encapsulation.\textsuperscript{32, 33, 34, 34b, 35, 19} The ability to manipulate and create numerous amphiphiles using similar building blocks allows them to be versatile in their applications.

1.4.1 Drug Delivery using Self-Assembled Nanostructures

The Cui group is one research group who used a peptide amphiphile template to create a modified structure, referred to as a drug amphiphile, for delivery of the anticancer drug, camptothecin.\textsuperscript{36} Often times, anticancer drugs are hydrophobic, making it difficult to use them for amphiphilic self-assembly. A short peptide sequence with a linker to the anticancer drug to create the amphiphilicity necessary to create a nanostructure with the drug embedded on the inside; the hydrophobicity of the drug drives the assembly, while the peptide sequence is the ultimate determinate in the final structure.\textsuperscript{36} Two different known β-sheet forming peptide sequences were used, GVQIVYKK (Tau) and NNQQNY (Sup35), for the hydrophilic portion of the amphiphile. Both used the same linker, buSS that utilizes a disulfide linker for reductive cleavage by glutathione; the linker was then attached to the camptothecin. The structures are shown in figure 1.4.\textsuperscript{36} By controlling the number of lysine units that were used in the
peptide sequence, one, two or four camptothecin units could be attached leading to drug loadings of 23%, 31%, and 38%, respectively. They discovered that mono-CPT using the Tau peptide sequence (mCPT-buSS-Tau) formed filamentous nanostructures with an typical width of 6.7 nm; the di-CPT of the same sequence (dCPT-buSS-Tau) formed nanofibers with an average width of 7.2 nm; the tetra-CPT using the Tau sequence (qCPT-buSS-Tau) formed shorter nanofibers with a width of 9.5 nm.\(^{37}\)

![Figure 1.4 Two drug amphiphiles used by Lock and coworkers for drug delivery.\(^{36}\)](image)

1.4.2 Encapsulation using Amphiphilic Self-Assembly

An excellent material for optoelectronics\(^{38}\) sensing\(^{39}\), electrical conductivity\(^{40}\), and mechanical applications\(^{41}\) is a carbon nanotube. Carbon nanotubes are extremely
insoluble and often aggregate in most solvents; therefore, they need some sort of functionalization to be useful for their potential applications.\textsuperscript{39,42,43} Ideally, the functionalization should not affect the properties of the carbon nanotubes, and should lead to stable, uniform assemblies.

Several research groups have explored the potential of using peptide conjugates for the encapsulation of carbon nanotubes.\textsuperscript{34b,44,45} Specifically, Arnold and coworkers used three different peptide amphiphiles to functionalize carbon nanotubes.\textsuperscript{34b} The hypothesis was that the hydrophobic portion of the peptide amphiphile would bind with the carbon nanotube leaving the hydrophilic peptide portion on the outside for sensing or other applications; the hypothesis was that this should not disrupt the carbon nanotube properties, and should be controllable by pH.\textsuperscript{34b} Ultimately, this approach was successful in the dispersion of carbon nanotubes in solution through the utilization of an amphiphile to non-covalently link with the carbon nanotubes; knowing this information, the peptide amphiphile used could contain some sort of bioactive material for further applications.\textsuperscript{34b}

1.5 NDI-lysine Amphiphilic Self-Assembly

Through the manipulation of noncovalent interactions for self-assembly, the Parquette group has developed peptide amphiphile derivatives to study different types self-assembly and the applications of nanostructures that form. For example, Shao and coworkers developed a π-conjugated, 1D nanostructure to be used for organic electronic devices.\textsuperscript{46,47} The nanostructure was created via beta-sheet assembly of a dipeptide, using a 1,4,5,8-naphthalenetetracarboxylic acid diimide in the ε-amino position of a hydrophilic dilylsine as a π-π stacking unit and hydrophobic side chain, shown in figure 1.5.
The peptide sequence was modified in water using acetyl groups to protect the free amines, or adjusting which lysine the NDI unit was attached to; by modifying the hydrophobic and hydrophilic portions of the amphiphile, Shao was able to achieve soluble 1D nanostructures with well-tailored properties. When dipeptide A was imaged using transmission electron microscopy (TEM), helical nanofibers with uniform diameters formed when dissolved in water. On the other hand, dipeptide B showed flattened, twisted nanoribbons when dissolved in water and imaged with TEM; in this case, the NDI unit was placed on the other lysine unit. In contrast to dipeptides A and B, dipeptide C showed increased repulsion due to the electrostatic interactions from the second free amine group, leading to no observed assembly at 250 µM using TEM.

Shao used UV-vis spectroscopy to observe the effects of π-π stacking of the NDI units during assembly. Shao reported using trifluoroethanol (TFE) to disrupt the π-π
stacking in peptide-dendron hybrids; TFE was also used in this study to analyze the effects of the NDI stacking compared to the stacking in pure water. The UV spectrum is shown in figure 1.6. It is important to note that a single NDI molecule contains two absorbances between 300 and 400 nm and at 240 nm, denoted as band I and II. All three peptides exhibited these bands on the UV-vis, however, for dipeptides A and B, band I decreased in intensity and exhibited a red shift, indicating that $J$-type $\pi-\pi$ interactions are the contributing factors in the nanostructure formation.

Figure 1.6 UV-vis spectrum of dipeptide A demonstrating a red shift from TFE to water.

1.6 References


Chapter 2: Decoration of an Amphiphile with Gold Nanoparticles

2.1 Introduction

Gold nanoparticles have been of great interest in research due to their chemical, optical, catalytic, biomedical, and electronic properties. Their properties can have potential applications in optics, sensing, nanoelectronics, catalysis, biotechnology, and medicine. The reactivity of the gold nanoparticles is largely dependent on the size and shape of the particle that is formed. Depending on the method of reduction of gold (III) to gold (0), variation of the size of the gold nanoparticle is possible; the size can range from 1-100 nm. If gold nanoparticles are used in catalysis, for example, their reactivity increases as surface area decreases; therefore, the smallest particles (1-2 nm) have the highest activity. The Turkevich method of reducing tetrachloroauric acid (HAuCl₄) to gold (0) with citric acid can be utilized to control the size of the nanoparticle growth. Several other methods have been used including reduction by ascorbic acid, sodium borohydride, sonochemistry, and photochemistry. Often times, a surfactant is used to stabilize the gold nanoparticles and prevent aggregation or growth in solution.

The Turkevich method is the most common way to reduce gold (III) to gold (0) using citrate as the reducing agent; the growth mechanism for this method was thought to be quick, yielding larger particles. Recently, however, Polte and coworkers have studied this mechanism in more detail; they determined that the mechanism is a result of
the fast reduction of gold (III) to gold (I), then the subsequent slower reduction to gold (0) to form the gold nanoparticles.\textsuperscript{62} Initially, particles are formed from small nuclei aggregating into larger particles until the gold (I) is completely consumed; this led to particles averaging a diameter of 16 nm.\textsuperscript{62}

Gold nanoparticles have been templated on several different types of stabilizing agents to control their shape and size, including polymers,\textsuperscript{64} inorganic particles,\textsuperscript{65} or assembled 1-D nanostrutures.\textsuperscript{66} There has been great success for gold nanoparticle formation using amine and thio- attractive functionalities as the template surfaces.\textsuperscript{67} The Parquette group has developed amphiphilic lysine-NDI molecules that contain a charged amine in the polar head that has the ability to attract anionic gold tetrachloride salts and gold nanoparticles that are stabilized by the citrate anion. This chapter will go into detail about lysine-NDI-diacetylene amphiphile assemblies capable of being templates for gold nanoparticles.

\section*{2.2 Research Design}

Gold nanoparticles are most commonly synthesized via the Turkevich method of formation, which reduces the strong acid tetrachloroaauric acid (HAuCl\textsubscript{4}) using sodium citrate in water at 100°C as shown in figure 2.1.\textsuperscript{68} There are two different ways the gold can interact with the cationic surface present; one option is that initially, when the HAuCl\textsubscript{4} is dissolved in water, it dissociates into a gold (III) tetrachloride anion that can interact with the cationic surface that is present through electrostatic interactions.\textsuperscript{69} The other way gold can interact with the cationic surface is as a gold nanoparticle coated in citrate, which is negatively charged.\textsuperscript{67} Once coated in citrate, the nanoparticles can
interact with the charged amine surface. Therefore, the interaction between gold and the cationic surface can be in the ionic or colloidal form.

Figure 2.1 The Turkevich method of reducing gold(III) to gold(0) (A). Two ways gold can electrostatically interact with cations; the non-reduced gold(III)tetrachloride anion or the reduced colloidal gold coated with citrate can interact with a positively charged surface, in this case amines (B).

To functionalize multi-walled carbon nanotubes (MWNT), Jiang and coworkers utilized the cationic polyethyleneamine (PEI) to wrap the tube’s surface with amine groups. Citric acid was used as the reducing agent for HAuCl₄, and it also helped with preventing aggregation of the modified MWNTs by protonating the amines creating an electrostatic repulsion between tubes. When the HAuCl₄ was added to a solution containing the modified MWNTs and citric acid, gold nanoparticles were formed and
they decorated the surface of the carbon nanotubes (Figure 2.2). Jiang also used heat treatment with ammonia to functionalize only the inside of carbon nanotubes with amine groups; using the same method of adding citric acid to the tubes, followed by HAuCl₄, the MWNTs were decorated on the inside with gold nanoparticles.

Figure 2.2 Carbon nanotube functionalized with amines interacting with citric acid to attract gold nanoparticles (A). MWNT functionalized with amines (B). Functionalized MWNT coated with gold nanoparticles (C).

Another example of gold nanoparticles coating the surface of a specific surfactant was an amphiphilic pillar[5]arene used by Yao and coworkers. The amphiphile pillar[5]arene can form micelle type structures in water that can transition to vesicles, or
vice versa, depending on the pH. However, when these structures were exposed to HAuCl₄ using ascorbic acid as the reducing agent, the pillar[5]arene acted as a stabilizing agent for the gold nanoparticles creating a microtube bilayer structure. They found that the amine rich amphiphile interacted with the gold nanoparticles via hydrogen bonding, absorbing them onto the surface of the tubes. Two methods were used to decorate the tubes with gold nanoparticles; one used the self-assembled tubes and immersed it into a solution of gold nanoparticles, while the other prepared the microtubes in the presence of gold nanoparticles. Interestingly, the tubes prepared in the presence of gold nanoparticles took two months to prepare, but they were much more stable under various physical conditions including pH and temperature; the gold nanoparticles more evenly dispersed, compared to just directly attaching the gold nanoparticles to the outside of the tubes. The structure of the pillar[5]arene and TEM images are shown in figure 2.3.
Peptide nanofibers can also be used as a template for gold nanoparticles by utilizing the gold-amine affinity in the peptide sequence, according to Acar et al. The
sequence used to form amyloid fibers was Ac-KFFAAK-NH₂, which exploits the \( \pi-\pi \) stacking ability of having two phenylalanine amino acids next to each other; this is not crucial to the formation of the structure but it does contribute. This sequence is a \( \beta \)-sheet forming peptide, with the amine groups on the exterior surface.\(^\text{71}\) Using ascorbic acid as the reducing agent for KAuCl₄, the peptide sequence Ac-KFFAAK-NH₂ assembled into nanofibers with gold nanoparticles on the outside of the fiber (figure 2.4).\(^\text{71}\) The one-dimensional gold nanostructures were achieved using the peptide as the template, and by adjusting the ratios of the reducing agent, the gold precursor, and the peptide Acar and coworkers were able to control the shape of the gold nanostructures, including nanowires, noodle shaped, and spherical gold nanostructures.\(^\text{71}\) The gold nanowire structures were capable of conduction between two gold surfaces with the fiber exhibiting tunnel-dominated conductance.\(^\text{71}\)
2.3 Research

Amphiphilic lysine-NDI molecules exhibit a polar head group containing an ammonium cation. One NDI amphiphile containing a diacetylene unit in the non-polar tail, displayed some robust characteristics toward conditional changes like pH change and temperature change, especially after polymerization. The π-π stacking of the NDI core and diacetylene tail contributes to the directional assembly, enforcing its robustness. In many cases, conditional changes drastically affect self-assembled systems, especially if
they lack intermolecular, non-covalent interactions like hydrogen bonding or hydrophobic interactions. Surfactants are often used to stabilize gold nanoparticles, coating the surface, therefore, affecting the assembly due the stronger interactions between the gold and the ligand. There a very few examples of amphiphilic assemblies being used as templates for gold nanoparticle growth; the surfaces that are mostly used are surfaces like carbon nanotubes, silica oxide, or another inorganic surface.65, 69 However, some self-assembling molecules with very strong non-covalent interactions have the potential to be stable enough to stabilize gold nanoparticles on their surfaces.71 Due to the strength of the stacking of the diacetylene unit and NDI chromophore in compound A, as well as its charged ammonium polar head, the nanotube assembly could provide an excellent surface for gold nanoparticle formation. Using a reducing agent like citric acid or citrate to reduce tetrachloroauric acid would also likely be successful due to the stability of the amphiphile to pH changes. With these conditions, citric acid can reduce the gold on the surface of compound A leading to an even decoration of gold nanoparticles on a stable surface (figure 2.5).
Figure 2.5 Amphiphilic compound A assembled and decorated with gold nanoparticles (Picture taken from the thesis of Nicholas Bewick).

2.4 Results and Discussion

Lysine NDI-amphiphiles exhibit a cationic ammonium head group that has the potential to attract and support gold nanoparticles to the surface of a nanotube or β-sheet. The amphiphile NH$_2$K-(NDIDA)-OMe (figure 2.5, A) provides the opportunity to act as a surfactant for gold nanoparticle formation. Compound A was able to form nanotube assemblies in neutral water at a concentration of 2 mM. To produce gold nanoparticles, citric acid was used as a reducing agent, but to confirm that it does not affect nanotube morphology, citric acid (2 mM, 1 eq, pH=3.4) was added to the solution of compound A. Citric acid lowered the pH slightly, but the structure of the nanotubes was not affected; the tubes looked very similar to those at pH = 7.0, based on TEM imaging. Tetrachloroauric acid (0.67 mM, 0.67 eq) was then added to examine the effects on the original nanotube; the pH did lower to 3.0, and spherical gold nanoparticles were seen on
the surface of the nanotube (Figure 2.6). Without the presence of compound A, non-uniform gold nanoparticles of varying shapes were seen, demonstrating the template ability of the nanotubes, similar to other stabilizing agents. In these conditions, the average diameter of the gold nanoparticles on the surface of the tube was 7.6 nm.

Figure 2.6 TEM image of 1 eq. of citric acid in solution of compound A (500 µM), followed by addition of tetrachloroauric acid (0.67 eq.) aged for 1 d at pH = 3.0 (A). TEM image on carbon-coated grid, not stained, of compound A (500 µM) with citric acid and tetrachloroauric acid (3:2, 500 µM citric acid) in water after 1 d of aging at pH = 3.3 (B). Sizes of observed gold nanoparticles with conditions described in B (C).

The order of addition proved to be critical in the formation of uniform gold nanoparticles on the surface of the nanotube. Based on previous studies of gold nanoparticles, the hypothesis of the interaction in this specific case, was that the tetrachloroauric anion would electrostatically interact with the charged ammonium group, coating the tube surface with citric acid then reducing the gold (III) into gold particles. Various orders of addition of the three reagents were tested to help confirm this hypothesis. When citric acid (1 mM, 1 eq.) was added to a solution of HAuCl₄ (667 µM,
0.67 eq) and compound A (1 mM, 1 eq.), the gold nanoparticles were irregular, and non-spherical (figure 2.7). Citric acid has been shown to reduce gold into gold nanoparticles in several reports; it is able to coordinate to the ammonium cation on the nanotube, with the surface providing stability for controlled gold nanoparticle growth. If the citric acid is added after the gold is added to the nanotubes, it will be more dilute making it less concentrated on the tube surface. This prevents controlled growth of spherical gold nanoparticles, leading to aggregation of the reduced gold into nanoclusters.

Figure 2.7 TEM images of compound A (500µM) and tetrachloroauric acid (333 µM) followed by addition of citric acid (500 µM).

To study the growth of gold nanoparticles using citric acid on the surface of compound A, a titration was performed using 1 mM tetrachloroauric acid and 500 µM citric acid, adjusting the concentration of compound A. As the ratio of gold (III) to
compound A increased to about 1:1, the diameter of the gold nanoparticles grew very quickly; at a ratio of 0.2, the gold nanoparticles had an average diameter of 8.4 nm, and when the ratio was 1.0, the average diameter of the gold nanoparticles was 22.5 nm (table 2.1). As the ratio went up to 3.2, the growth of gold nanoparticles stabilized, likely due to the lack of concentrated citric acid reducing agent. By the time the ratio reached 3.2, the concentration of compound A was diluted to 60 µM and the tubes started to dissipate, indicating the critical micelle concentration had been reached. At this point, the gold nanoparticles had reached a diameter of 29.5 nm, a significant increase even from the higher ratio titrations. In the experiments with a ratio of 1.0 to a ratio of 1.6, the diameter of the particles only increased by 1.4 nm, but up to a ratio of 3.2 it jumped another 5.4 nm. When the image was examined closely, the amphiphile could be seen coating the nanoparticles.
Table 2.1 Titration of compound A (500 µM) and citric acid (500 µM) with HAuCl₄ (1 mM).

<table>
<thead>
<tr>
<th>Ratio of Gold to Citric Acid</th>
<th>0.2</th>
<th>0.4</th>
<th>0.6</th>
<th>0.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration of Compound A and Citric Acid (µM)</td>
<td>417</td>
<td>357</td>
<td>308</td>
<td>268</td>
</tr>
<tr>
<td>Average Diameter of the Gold Nanoparticles (nm)</td>
<td>8.4</td>
<td>9.5</td>
<td>16.9</td>
<td>21.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ratio of Gold to Citric Acid</th>
<th>1.0</th>
<th>1.2</th>
<th>1.6</th>
<th>3.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration of Compound A and Citric Acid (µM)</td>
<td>233</td>
<td>204</td>
<td>158</td>
<td>60</td>
</tr>
<tr>
<td>Average Diameter of the Gold Nanoparticles (nm)</td>
<td>22.5</td>
<td>22.8</td>
<td>23.9</td>
<td>29.5</td>
</tr>
</tbody>
</table>

To confirm this observation, tetrachloroauric acid (5 mM, 10 eq.) was added to compound A: citric acid (1:1, 500 µM) solution resulting in a 250 µM concentration of compound A, which acts as the surfactant. The nanotube concentration in this case is high enough that the tubes should not be affected by the concentration of gold. The TEM images showed gold nanoparticles between 6 and 25 nm in diameter evenly coating the
outside of the nanotube. The images showed defined edges of the nanogold without noticeable surfactant coating (figure 2.8).

![Figure 2.8 TEM images of compound A decorated with gold nanoparticles in water (250 µM, 10 eq. HAuCl₄, and 250 µM citric acid) (A and B).](image)

Gold nanoparticles have shown an affinity for diacetylene functionality and diacetylene has been known to coordinate metals.⁷⁵ Since compound A has a diacetylene unit, coordination is possible with the gold. Therefore, to confirm the electrostatic interaction between the charged ammonium group on compound A with citrate coating as the source of gold nanoparticle decoration, and not diacetylene coordination, tetrachloroauric acid was reduced using the assembled nanotubes NH₂-Lys(NDI-nBu)-OMe (compound B) as the surfactant with citric acid. Under the control conditions using citric acid (500 µM), and HAuCl₄ (333 µM), these nanotubes were not stable. To
determine if the pH was a factor, sodium citrate was used to replace citric acid; this caused the pH to increase to 5.2. The shape and size of the tube under these conditions was different than the assembly at pH 7, but short and wide nanotubes, with a diameter of 100 nm, did form and they were decorated with gold nanoparticles (figure 2.9 A and B). This confirms the hypothesis that the charged ammonium head group of the amphiphile is responsible for the electrostatic interactions with the gold nanoparticles leading to decoration of the outside of the tube. The gold nanoparticles evenly decorated the outside of compound B with an average diameter ranging between 9 and 13 nm.

Figure 2.9 TEM images using the amphiphile NH$_2$-Lys(NDI-$n$Bu)-OMe (compound B, 500 µM), sodium citrate (1 eq.), and HAuCl$_4$ (0.67 eq.) in water after 1 day of aging.

Using the citrate conditions on compound A (1:1 sodium citrate, 0.67 eq. HAuCl$_4$) instead of citric acid, the nanotubes retained their size and shape, with even
dispersion of the gold nanoparticles. The decoration on the surface of the tubes was better than the samples using citric acid (figure 2.10), with nanoparticle diameters averaging 9 to 13 nm. In this case, the pH seemed to be playing a factor in the uniform growth of the gold nanoparticles on the surface of the tube, which has been seen with other research on decoration of templates using gold nanoparticles.\textsuperscript{67} Since citrate is negatively charged, it will be more likely to electrostatically interact with the positive nanotube surface compared to neutral citric acid. Mono- and disodium citrate have a pKa of 4.77 and 5.19 respectively, while citric acid has a pKa of 3.15. As the solution increases in basicity, the more favorable sodium citrate is, which increases the overall negative charge, increasing the attraction to the surface of the tube. This leads to more interaction of reducing agent on the outside of the tube, resulting in more gold nanoparticle growth on that surface and more even dispersion.
The purpose of using different templates for gold nanoparticles is to prevent agglomeration of the gold, and to increase its stability. A solution of HAuCl₄ (0.67 mM) in water was added to a solution of compound A (1 mM) and sodium citrate (1 mM); the resulting solution was aged for up to 7 days (figure 2.11). After 7 days of aging, there was no change in the gold nanoparticles decorating the surface of the nanotube, with no aggregation. Each day between 1 day and 7 days, the sample was imaged, with the gold nanoparticles ranging in diameter between 12.0 and 14.4 nm. Since there was no aggregation or change in the tubes over the course of 7 days, the nanotubes do indeed stabilize the gold nanoparticles.
Figure 2.11 TEM images of compound A (500 µM), with sodium citrate (500 µM) and HAuCl₄ (333 µM) aged for 1 day (A), 3 days (B), and 7 days (C).

Due to their surface plasma oscillations, gold nanoparticles are capable of absorbing UV light. The wavelength the nanoparticle absorbs is directly related to its size. When compound A was dissolved in just water, absorptions corresponding to the NDI chromophore π-π* band I (330 nm to 390 nm) and band II (240 nm to 270 nm) were observed; similar peaks were found when compound B was dissolved in water without any gold present (figure 2.12). Also, if tetrachloroauric acid was dissolved in water with reducing agent present (e.g. sodium citrate 500 µM), after 20 h a new peak appeared at 525 nm (figure 2.12), a characteristic peak for gold nanoparticle formation. To show that no other peaks were observed in this region, the UV of both compound A and compound B were taken with just reducing agent present; compound A showed no change with both citric acid and citrate, while compound B showed a difference between the two reducing agents. This is likely due to the fact that no tubes were observed for compound B when citric acid was used; this is why the two UV spectra look different for compound B depending on reducing agent. When a UV was taken using the control
conditions of compound A or B (500 µM) with reducing agent (500 µM) and HAuCl₄ (333µM) the peak for gold nanoparticles was observed at 550 nm indicating a red shift. Therefore, the gold nanoparticles on the surface of the tube are slightly different then those formed only in the presence of only reducing agent. This observation is clearly indicated using compound B, due to the lack of polymerization; since the diacetylene in compound A has the capability to polymerize in light, there is a slight peak there representing the diacetylene polymer that absorbs in the same region as the gold (0).
Figure 2.12 UV of gold (III) reduced with sodium citrate after 0 h (red) and 24 h (blue) (A). UV of compound A dissolved with citric acid (red) and citrate (blue) in water (B). UV vis spectrum of compound B dissolved with citric acid (red) and citrate (blue) in water (C). UV vis spectrum of compound B dissolved with citrate (1 eq) and gold (III) (0.67 eq) after 0 h (red) and 48 h (blue) (D).

Compound A can transform from nanotube assemblies to sheets and helical coil assemblies, by heating compound A in a pressure tube to 130 °C for 30 minutes. The sheets were also used to template the gold nanoparticles under several conditions using
sodium citrate and citric acid as the reducing agent. The control conditions did show gold nanoparticle formation, but slightly different conditions showed more evenly coated sheets with gold nanoparticles. To a solution of compound A (1 mM) and citric acid (2 mM), HAuCl₄ (0.667 mM) was added and aged for 12 h. These conditions showed the best dispersion of gold nanoparticles on the surface of the sheets compared to the control conditions using a lower concentration of reducing agent (figure 2.13), and showed citric acid is slightly better than citrate for the reducing agent at coating the surface evenly. All conditions showed gold nanoparticle growth on the surface of the sheets, but 1: 2: 0.67 amphiphile to citric acid to gold showed the best coating. The average gold nanoparticle size was similar to the nanotube gold decoration, averaging in diameter from 5.3 to 7.9 nm, with some larger particles with a diameter of 14.8 nm.
Figure 2.13 TEM image of compound A (500 µM), citric acid (500 µM) and HAuCl₄ (333 µM) (A). TEM image of compound A (500 µM), citrate (500 µM) and HAuCl₄ (333 µM) (B). TEM image of compound A (500 µM), citric acid (1 mM) and HAuCl₄ (333 µM) (C). TEM image of compound A (500 µM), citrate (1 mM) and HAuCl₄ (333 µM) (D).

Compound A is capable of quick polymerization when exposed to light due to its diacetylene unit. The polymerized assembly of compound A has shown more stability than the non-polymerized assembly under various pH conditions and temperature
changes, similar to other diacetylene polymer assemblies.\textsuperscript{77,78} The polymerization can be tracked and observed via UV absorptions, at 500 nm and 650 nm;\textsuperscript{79} when a solution of compound A (500 µM) was exposed to UV light (254 nm, 19 W), the solution went from pale pink to dark purple, and showed UV absorptions at 593 nm, 540 nm, and 504 nm after 30 minutes (figure 2.14). This corresponds to known absorbances for diacetylene UV induced polymerization. Polymerization could also be done on the nanosheets that were formed using the heat treatment at 130 °C in a pressure tube for 30 minutes. After irradiation for 30 minutes, this solution turned from pale pink to red. Strong absorptions were seen on the UV at 540 and 504 nm (figure 2.14), but the 593 nm absorbance disappeared. Previous reports have shown that the dark purple color emerges from the absorance at 593 nm.\textsuperscript{80}
The polymer of compound A still contains the charged ammonium head group that can attract reducing agent to reduce gold nanoparticles, so it was hypothesized that it could also act as a surfactant for gold nanoparticles. By using various concentrations of reducing agent and tetrachloroauric acid, the ideal conditions were found to be the same as for the non-polymerized tubes. TEM images showed even dispersion of gold nanoparticles on the surface of the polymerized nanotube structures when polymerized compound A (1 mM) was mixed with citric acid or citrate (1 mM) and HAuCl₄ (0.667 mM) and aged for 12 h (figure 2.15). The nanoparticles ranged in diameter from 4.2 nm to 19.0 nm under the conditions using citrate, and 15.1 nm to 24.5 nm using the same conditions with citric acid.
Figure 2.15 TEM image after induced polymerization of compound A (500 µM), using UV light (254 nm, 19 W), with citric acid (500 µM), and HAuCl₄ (333 µM) (A); TEM image after induced polymerization of compound A (500 µM), using UV light (254 nm, 19 W), with citrate (500 µM), and HAuCl₄ (333 µM) (B).

Again, the purpose of the template for gold nanoparticles is to prevent aggregation, and increase stability of the gold nanoparticles. The polymerized compound A (1 mM) with sodium citrate (1 mM) was combined with HAuCl₄ (0.67 mM) and the solution was aged for up to 7 days (figure 2.16). After 7 days of aging, the gold nanoparticles did not aggregate and evenly coated the outside of the tubes. The nanoparticles averaged in diameter between 4.2 and 19.0 nm.
The final template used for gold nanoparticle growth with this template was the polymerized amphiphile that forms sheets. Before polymerization, the sheets were made by heating a solution of compound A (2 mM) in water in a pressure tube at 130 °C for 30 minutes. After the sheets were made, polymerization occurred by shining UV light (254 nm, 19 W) on the solution for 30 minutes, creating a dark red solution. This solution was used as the template for gold nanoparticles (1 mM), using sodium citrate or citric acid (1 mM) as the reducing agent for HAuCl₄ (0.67 mM); the resulting solution was aged for 12 h. These conditions again showed excellent, uniform growth of gold nanoparticles on the surface of the sheets (figure 2.17). The nanoparticles ranged in diameter from 14 to 20 nm using sodium citrate as the reducing agent.
Figure 2.17 TEM images of polymerized sheets of compound A by heating compound A (2 mM) to 130 °C for 30 minutes, then shining UV-light (254 nm, 19 W) on the resulting solution for 30 minutes; TEM image of compound A (500 µM), citrate (500 µM), and HAuCl₄ (333 µM) in water after 12 h (A); TEM image of compound A (500 µM), citric acid (500 µM), and HAuCl₄ (333 µM) in water after 12 h (B).

2.5 Conclusion

Using an NDI diacetylene compound as a template for gold nanoparticle growth proved to be a successful strategy, using citric acid as the reducing agent initially. However, when sodium citrate was used the gold nanoparticles were more evenly dispersed on the surface of the tube, compared to citric acid; this is likely due to the slight increase in pH when using citrate versus citric acid. The gold nanoparticles showed a slight red shift to 550 nm in the UV when on the surface of the tube, compared to in a solution with only reducing agent. When comparing the two NDI structures, one with a diacetylene tail (compound A) and one with an n-butyl tail (compound B), compound A showed to be more stable under the conditions for gold nanoparticle growth. The stability could be due to the increased π-π stacking ability of the diacetylene units, which
compound B lacks. Due to the more robust characteristics of compound A after self-assembly, including its ability to polymerize, further locking the structure in place, they have the potential to be a platform for gold heterogeneous catalysis. These nanotubes are stable to elevated temperature and pH conditions, giving them the potential to be used in various sets of reaction conditions. Overall, the various structure types and reaction conditions using compound A to template gold nanoparticles has been successful.

2.6 Methods and Experimentalss

**General Methods**

Transmission electron microscopy (TEM) was performed with Technai G2 Spirit instrument operating at 80 kV. All reactions were performed under argon or nitrogen atmosphere, unless otherwise indicated. Solution-state $^1$H and $^{13}$C NMR spectra were recorded on Bruker DPX-250, DPX-400, or DPX-500 instrument as indicated. Dimethylformamide (DMF) was dried by distillation from MgSO$_4$. Chromatographic separations were performed on silica gel 60 (230-400 mesh, 60 Å) using the indicated eluents. HPLC was done with reverse-phased preparative Waters XBridge C8 column eluting with a linear gradient of CH$_3$CN/water (20/80 to 100/0 over 40 minutes, 0.1 % TFA). Purity was assessed by analytical reverse-phase HPLC. All water used for sample solutions was HPLC grade and passed through membrane filter (0.02 µm) before use.

**Transmission Electron Microscopy Measurements**

10 µL of solution was applied to a carbon-coated copper grid (Ted Pella, Inc.) for 2 min with no uranyl acetate negative stain. The dried sample was imaged using a
Technai G2 Spirit TEM instrument operating at 80 keV, and the data obtained was analyzed using Image pro software.

**General Gold Decoration Procedure**

The amphiphile was dissolved in water (10 mM, pH=7) and aged for 12 h at room temperature. After 12 h this solution was diluted to 2 mM. Sodium citrate or citric acid was then added to the amphiphile solution (1:1 amphiphile : citrate, 2 mM), giving a 1 mM concentration of nanotubes coated with reducing agent. Tetrachloroauric acid (0.667 mM) was added to the solution, diluting again to half the original concentration, giving an amphiphile: citrate: gold ratio of 1: 1: 0.67 (500 µM, 500 µM, 333 µM). This solution was aged for an additional 12 h before preparing TEM samples.

\[ n\text{-Bu-NDA 2.1}^{73} : \text{1,4,5,8 Naphthalenetetracarboxylicdianhydride (9.65 g, 35.9 mmol)} \]

was dissolved in DMF (300 mL) and degassed with nitrogen gas. This solution was heated to 140°C; a solution of \( n \)-butylamine (3.5 mL, 2.59 g, 35.3 mmol) in DMF (50 mL) was added dropwise via an addition funnel over 2 hours. The solution was heated to
reflux and stirred for 18 hours, then cooled to room temperature and placed in the freezer for 1 hour. Precipitate was filtered off, and the filtrate was condensed in vacuo. The remaining residue was purified with flash column chromatography (CHCl₃) to yield a yellow solid 2.1 (1.51 g, 13%). ¹H NMR (250 MHz, CDCl₃) δ 1.00 (t, J = 3.6 Hz, 3H), 1.46 (m, 2H), 1.73 (m, 2H), 4.21 (t, J = 3.9 Hz, 2H), 8.82 (s, 4H).

Boc-K(NDI-nBu)-OH 2.2: Compound 2.1 (1.51 g, 4.48 mmol) was combined with Boc-Lys-OH (1.1075 g, 4.38 mmol) in DMF (110 mL). The solution was heated to 125°C under nitrogen gas, and was stirred for 16 hours. DMF was removed via vacuum distillation. The compound was purified via flash column chromatography (CHCl₃, 1%-10% MeOH in CHCl₃) to yield compound 2.2 as a yellow solid (1.21 g, 2.19 mmol, 53%). ¹H NMR (250 MHz, CDCl₃) 0.99 (t, J = 3.7 Hz, 3H), 1.35-1.55 (m, 13H), 1.68-1.79 (m, 5H), 1.97 (m, 1H), 4.20 (t, J = 3.8 Hz, 4H), 4.30 (m, 1H), 8.74 (s, 4H).
**Boc-K(NDI-nBu)-OMe 2.3:** Compound 2.2 (0.30 g, 0.55 mmol) was dissolved in DMF (1.2 mL). Potassium carbonate (0.226 g, 1.6 mmol) and methyl iodide (0.103 mL, 0.23 g, 1.6 mmol) were both added to the mixture. The reaction stirred for 18 h. The remaining residue was dissolved in ethyl acetate and wased with water (3 x 75 mL). The organic layer was collected and dried with Na₂SO₄. The solvent was removed in vacuo, and the remaining solid was purified via flash column chromatography (0.5% MeOH in CHCl₃) to give a yellow solid Boc-K(NDI-nBu)-OMe 2.3 (0.14 g, 0.24 mmol, 44%).
**NH₂-K(NDI-nBu)-OMe B**<sup>73</sup>: Compound **2.3** (0.78 g, 1.4 mmol) was dissolved in 95:5 TFA:TES (1 mL). The solution was left for 2 h to stir in a capped vial. The solvent was removed via blowing nitrogen over the surface of the solution. The remaining solvent was dissolved in water and acetonitrile and purified via reverse phase HPLC to yield NH₂-K(NDI-nBu)-OMe (0.38 g, 0.82 mmol, 58%).

**Propargyl-NMA 2.5**: 1, 4, 5, 8-naphthalenetetracarboxylic dianhydride (5.00 g, 18.6 mmol), water (800 mL), and potassium hydroxide (88 mL, 1.0 M) were combined in a round bottom flask. The mixture was stirred until the solid dissolved, and the pH was adjusted to 6.3 using phosphoric acid (1.0 M). To the solution, propargylamine (1.03 g, 18.7 mmol, 1.20 mL) was added and acidified with phosphoric acid (1.0 M) to a pH of
2.0 to yield a white precipitate. The precipitate was filtered, washed with water, and dried in a dessicator under vacuum. When dry, this solid was suspended in acetic anhydride (150 mL) and heated to 110 °C for 16 h. The solvent was removed in vacuo to yield the monoanhydride 2.5 (4.40 g, 14.4 mmol, 77%). $^1$H NMR (250 MHz; DMSO): $\delta$ 8.73 (s, 4H), 4.82 (d, $J$ = 2.1, 2H), 3.20 (t, $J$ = 2.1, 2H), 3.20 (t, $J$ = 2.1, 1H).

Boc-K(NDIMA)-OH 2.6: The monoanhydride 2.5 (4.40 g, 14.4 mmol) and Boc-Lys-OH (3.55 g, 14.4 mmol) were combined and dissolved in DMF (350 mL) and stirred under nitrogen. The mixture was heated to 120 °C for 14 h resulting in a dark brown solution. Vacuum distillation was used to remove DMF. The solid was purified via column chromatography using silica gel (CHCl$_3$ to 2% MeOH in CHCl$_3$) yielding a yellow amino acid 2.6 (6.38 g, 12.0 mmol, 83%). $^1$H NMR (250 MHz; CDCl$_3$) $\delta$ 8.81-8.74 (m, 4H), 5.16 (dd, $J$ = 6.6, 0.3, 1H), 4.97 (d, $J$ = 2.4, 2H), 4.29 (s, 1H), 4.20 (t, $J$ = 7.3, 2H), 2.23 (t, $J$ = 2.5, 1H), 2.00-1.89 (m, 1H), 1.86-1.75 (m, 3H), 1.59-1.51 (m, 2H), 1.43 (s, 9H).
**Boc-K(NDIMA)-OMe 2.7**: Boc-K(NDIMA)-OH (0.492 g) was dissolved in DMF (2 mL) under nitrogen. Potassium carbonate (0.382 g), and methyl iodide (0.172 mL, 0.393 g) were added. The mixture stirred for 14 h at room temperature. The remaining solid was dissolved in ethyl acetate, and washed with water (5x50 mL). The organic layer was dried over Na₂SO₄, and the solvent was removed in vacuo. The crude solid was purified by column chromatography (0.5% to 1% MeOH in CHCl₃) to yield an off white solid 2.7 (0.265 g, 52%). ¹H NMR (500 MHz; CDCl₃): δ 8.79 (q, J = 8.6, 4H), 5.08 (d, J = 8.0, 1H), 4.98 (d, J = 2.4, 2H), 4.30-4.29 (m, 1H), 4.22-4.19 (m, 2H), 3.74 (s, 3H), 2.24-2.23 (m, 1H), 1.93-1.85 (m, 1H), 1.81-1.71 (m, 3H), 1.56-1.44 (m, 2H), 1.42 (s, 9H).
**Boc-K(NDIDA)-OMe 2.8:** Compound 2.5 (0.175 g, 0.320 mmol) was dissolved in dichloroethane (2 mL) with 1-hexyne (420 µL) and piperdine (107 µL). Copper acetate (6 mg) was added. The flask was covered in aluminum foil and stirred open to air for 14 h. The solvent was evaporated using nitrogen gas, and the remaining solid was dissolved in chloroform. The crude product was purified via column chromatography (0.5% MeOH in chloroform), to yield 2.8 (0.1456 g, 72%). $^1$H NMR (250 MHz; CDCl3): $\delta$ 8.78 (d, $J = 16.8$, 4H), 5.09 (q, $J = 10.2$, 1H), 5.02 (s, 2H), 4.30 (q, $J = 7.0$, 1H), 4.20 (t, $J = 6.8$, 2H), 3.74 (s, 3H), 2.22 (t, $J = 6.8$, 2H), 1.89-1.68 (m, 5H), 1.60-1.58 (m, 2H), 1.49-1.45 (m, 3H), 1.43 (s, 9H), 0.86 (t, $J = 7.2$, 3H).
**NH₂-K(NDIDA)-OMe 2.7:** Compound 2.6 (70 mg, 0.11 mmol) was dissolved in 95:5 TFA:TES in a capped amber vial. The solution stirred for two hours, and TFA:TES was removed using nitrogen gas. The resulting solid was dissolved in water and acetonitrile and purified via reverse-phase HPLC. The collected fractions were lyophilized to give a white-pink powder A (43 mg, 0.082 mmol, 73%). $^1$H NMR (500 MHz; DMSO): $\delta$ 8.62 (q, J = 9.4, 4H), 4.97-4.89 (m, 1H), 4.89 (s, 1H), 4.03 (q, J=7.0, 2H), 3.74 (s, 3H), 2.28 (t, J = 6.9, 2H), 1.87-1.85 (m, 2H), 1.74-1.64 (m, 2H), 1.52-1.47 (m, 1H), 1.45-1.35 (m, 3H), 1.32-1.29 (m, 2H), 0.82 (t, J = 7.3, 3H).

**Polymerization of compound A:** A 0.1 cm UV quartz cell was charged with compound A in water (2 mM). The cell was irradiated in a dark, sealed container using a UV lamp (19 W) at a distance of 12 cm from the cell for 30 min. The pale pink solution turned purple.
Formation of sheets of compound A: Compound A (1.0 mg) was dissolved in HPLC grade water (0.948 mL) and placed in a pressure tube. The tube was covered in aluminum foil and the solution was heated at 130°C for 30 min.

2.7 References


Chapter 3: Controllable, stimuli-responsive assembly of anti-parallel β-sheet forming nanofibers

3.1 Introduction

In recent decades, stimuli responsive assemblies have peaked the interest of many research groups for the development of smart materials. Often times, these materials will respond to various chemical and physical environments by undergoing structural transformations; the responsiveness of these materials makes them incredibly useful for applications in optoelectronics, drug delivery, and catalysis. These types of assemblies can also be used to mimic nanoscale technology already observed in natural systems, including systems that control iridescence, protein functionalization, enzymes, and molecular switching. Nanotechnology and self-assembled materials provide a unique set of compounds that use simple subunits that can organize into highly ordered systems. Making self-assembling molecules adaptive to various external stimuli including pH, temperature, ligands, or light, would be extremely useful in the development of useful materials.

A reliable method for controlling self-assembled structures is by utilizing metal-ligand coordination chemistry using metalloporphyrins type molecules. For example, zinc porphyrins have been widely studied as self-organizing molecules that prove to have successful assemblies with the addition of an external nitrogen donor to form intermolecular Zn-N coordinations. Simple porphyrin π-π stacking interactions are
very strong, but they often lead to nonspecific aggregation in solution. However, the addition of a nitrogen containing ligand, can contribute to additional intermolecular interactions that assist with a more organized assembly, including electrostatic interactions, π-π stacking, hydrogen bonding, and coordination chemistry to form various types of nanostructures including nanotubes, nanorods, or micelles.

The Parquette group has developed a set of stimuli responsive tetrapeptide structures with the sequence Phe-Lys-Lys(TPP)-Lys, as well as their isotopic analogues using a 13C analogue of phenylalanine using Fmoc-solid phase technology. When dissolved in chloroform, these tetrapeptide sequences can assemble into β-sheet structures. Due to the tetraphenylporphyrin (TPP) unit on the second lysine amino acid, DABCO and pyridine were added to the solution to assist in the self-assembly mechanism as nitrogen donor ligands; both DABCO and pyridine have been known to coordinate Zn-TPP complexes. The addition of the ligands showed changes in the nanostructures, indicating a stimuli responsive quality of the tetrapeptides. Also, the addition of a disrupting agent, in this case the strong organic acid TFA, can reverse the ligand coordination to the Zn-TPP complex, while the reintroduction of the DABCO ligand drives the assembly process again. This system of tetrapeptides demonstrates a stimuli-responsive, versatile system that shows the reversible interconversion between various nanostructures dependent on the conditions.

The Phe-Lys-Lys(TPP)-Lys system of tetrapeptides form β-sheet structures when assembled. Parallel and anti-parallel β-sheets are difficult to distinguish, and there have been several types of spectroscopic, theoretical, and vibrational methods used to attempt to differentiate the two. Recently, IR absorption has been used to distinguish
between parallel and anti-parallel β-sheets by comparing the amide I band of a peptide sequence with its respective isotopic equivalent. The manipulation of anti-parallel amyloid β-sheet fibers is key in studying the impact of these types of structures on several neurodegenerative diseases, as well as applications for tissue engineering. This chapter will go into detail about the properties of the stimuli responsive, anti-parallel β-sheet forming tetrapeptides.

### 3.2 Research Design

In this work, two tetrapeptides were synthesized using Fmoc-solid phase synthesis, with the amino acid sequence Fmoc-Phe-Lys-Lys(PP)-Lys-NH₂ (figure 3.1). By using functional peptide conjugates as the molecular building block, highly defined nanostructures can occur by controlling the balance of the interactions holding the assembly together, like electrostatic repulsion, hydrophobic interactions, and π-π stacking. The sequence was hypothesized to form β-sheets due to the alternating hydrophilic and hydrophobic amino acids in the sequence (figure 3.1). The difference between the two tetrapeptide sequences synthesized is the difference in the lysine unit adjacent to the phenylalanine; one tetrapeptide has a free amine on the side chain (B), while the other has an acyl protecting group on the side chain (A). The acyl group was added to increase solubility, and decrease the unfavorable electrostatic interactions with the other amine group.
3.2.1 Metal-ligand coordination assists assembly process

The coordination of metal to porphyrin complexes has been widely studied as a way to control the stacking ability of zinc-porphyrin systems. Nitrogen containing ligands have been extensively studied in the organization of zinc porphyrin complexes; the nitrogen containing ligands coordinate to the zinc, creating a series of intermolecular Zn-N pentavalent coordinations. Without the use of a nitrogen containing ligand, porphyrins often stack via π-π interactions creating nonspecific aggregations in solution. The spontaneous formation of well-defined nanostructures is observed upon
addition of the nitrogen donor, using a combination of noncovalent, intermolecular interactions, including electrostatic interactions, metal-ligand coordination, hydrogen bonding, and π-π stacking interactions. Many assemblies using porphyrin arrays have been reported to form J- and H-aggregates of 2-D and 3-D nano-assemblies. The use of two nitrogen containing ligands is reported in this work, including 1,4-diazabicyclo[2.2.2]octane (DABCO) and pyridine.

3.2.2 Anti-parallel vs. Parallel β-Sheet IR studies

In the past several decades, the ability to distinguish between parallel and anti-parallel β-sheets has been studied using various electronic, vibrational, and theoretical spectroscopic methods. Rashba developed a method to distinguish the two types of β-sheets by showing that transition dipole coupling between isotopically labeled peptides can be determined by IR spectroscopy (figure 3.2). However, it is still difficult to distinguish between these two types of β-sheets in a concrete way. Recently, the analysis of naturally occurring peptide sequences and their isotopic derivatives have been studied using the amide I band in the IR spectra. The accuracy of these conformational assignments using the line shape analysis in the IR spectra is questionable for several reasons. A widely known example is the aggregated forms of the amyloid β-proteins that aggregate in Alzheimer’s disease show the widely split amid I band that often indicate anti-parallel β-sheets. However, NMR data could be used to provide extensive evidence proving that these proteins actually consist of parallel β-sheets. Also, there is not a simple model system for parallel and anti-parallel β-sheets; therefore, the sensitivity of the split amide I band for both parallel and anti-parallel β-sheets, as well
as the specificity of the amide I band in the presence of other types of secondary structure has not been studied.

Developing peptides, or using naturally occurring peptides that selectively assemble into parallel or anti-parallel β-sheets are often difficult to obtain and can be expensive to synthesize an isotopic derivative. A reliable method to observe and study these differences is key in understanding many types of neurodegenerative diseases, and would assist in the development of tissue engineering applications. It is widely accepted that the amide I bands characteristic for anti-parallel β-sheets show a strong band at 1628 cm⁻¹ and a weaker band at 1679 cm⁻¹. Parallel β-sheets have a slightly

Figure 3.2 Exciton band structures and IR spectra of anti-parallel and parallel β-sheets with their differences highlighted in the red box.¹⁰⁸
different splitting frequency in the amide I splitting; in simulations done by Axelsen et. al. the splitting for parallel β-sheets has a slightly lower wavelength than the anti-parallel sheets.\textsuperscript{109} This is due to the stretching of the carbonyl of the labeled amino acid; if there is a \textsuperscript{13}C in the carbonyl, it changes the vibration frequency due to the difference in reduced mass. Therefore, the parallel sheets show a more significant difference in the wavelength due to the fact that they are in much closer proximity than when the sheets are anti-parallel to each other, making the frequency difference much more pronounced. The more significant change between having two \textsuperscript{12}C adjacent to each other versus two \textsuperscript{13}C adjacent to each other is shown by a wider splitting and lower wavelength of the second peak in the amide I bands.\textsuperscript{109} Although the difference in the two types of sheets is subtle, it is enough to make a prediction as to which type is taking place.

\section*{3.3 Results and Discussion}

The tetrapeptides, \textit{Fmoc-FKK(TPP-Zn)K-NH\textsubscript{2} (compound 3.1)}, and \textit{Fmoc-FK(Ac)K(TPP-Zn)K-NH\textsubscript{2} (compound 3.2)}, as well as their isotopic derivatives \textit{Fmoc-\textsuperscript{13}FKK(TPP-Zn)K-NH\textsubscript{2} (compound 3.3)} and \textit{Fmoc-\textsuperscript{13}FK(Ac)K(TPP-Zn)K-NH\textsubscript{2} (compound 3.4)} were soluble in chloroform. Nanostructures formed in chloroform, but the overall structure could be changed using the nitrogen containing ligands DABCO and pyridine. The tetrapeptides used could interconvert between various types of structures in a reversible fashion depending on the conditions.

Initially, UV-vis was used to analyze the tetrapeptides using trifluoroethanol (TFE) and chloroform as the solvent. A previous study by Shao \textit{et. al.} showed that TFE disrupts the π-π stacking and thus the assembly process.\textsuperscript{48} This can be used as a control for the monomer spectrum for UV-vis and CD studies. The two tetrapeptides were each
dissolved in TFE, and the UV spectrum of both compound 3.1 and 3.2 showed a sharp Soret peak at 408 nm and two Q-band peaks at 530 and 567 nm, characteristic of the Zn-porphyrin chromophore. The CD spectrum revealed a flat line in TFE for both tetrapeptides, indicating a lack of observable chirality in the supramolecular assembly of the peptide conjugates. When both compounds 3.1 and 3.2 were dissolved in chloroform, a red shift was observed in the UV in both the Soret and Q-bands of the Zn-TPP chromophore, compared to TFE, indicating J-type aggregation. Therefore, the porphyrin-porphyrin stacking is aligned end-to-end, leading to π-π stacking interactions between the Zn-TPP conjugates, as well as other intermolecular interactions like hydrogen bonding, leading to 1-D nanostructures in chloroform.

In chloroform, the UV-vis spectrum of compound 3.2 (500 µM) produced a broad Soret band in the range of 380 to 450 nm, centering at 430 nm, as well as Q-band peaks at 563 and 604 nm. Compound 3.1 (500 µM) in chloroform showed a broad Soret peak, as well, between 400 to 440 nm, with two Q-band peaks at 563 and 604 nm. When DABCO was added (1 eq) in chloroform to compound 3.2, the Soret band centered at 430 nm shifted to 424 nm, indicating a blue shift, with a distinct shoulder at 413 nm; the Q-bands also shifted to 561 and 602 nm, also indicating a blue shift. On the other hand, when DABCO was added to compound 3.1, both the Soret band and the Q-bands exhibited a blue shift at 424 nm, and 563 and 602 nm respectively (figure 3.3).

The other ligand used to probe the assembly process for these compounds was pyridine. Pyridine (2 eq) was added to a solution of compound 3.1 in chloroform; the UV spectrum showed a blue shift in the Soret peak at 427 nm, as well as the Q-bands at 561 and 602 nm. When pyridine (2 eq) was added to compound 3.2, a blue shift was also
observed in both sets of peaks; the Soret peak shifted to 426 nm, while the Q-bands shifted to 558 and 601 nm (figure 3.3)

![Figure 3.3](image)

Figure 3.3 . UV-vis spectra of compound 3.1 (250 µM) in CHCl₃ with no ligand (red), DABCO (1 eq, blue), or pyridine (2 eq, green) (A); UV-vis spectra of compound 3.2 (250 µM) with no ligand (red), DABCO (1eq, blue) or pyridine (2 eq, green) (B).

Both compound 3.1 and 3.2 were titrated with both DABCO and pyridine, up to three equivalents, in chloroform. Using compound 3.1, titration with DABCO could be probed using UV spectroscopy; the spectrum showed an H-aggregate in the Soret band, with a shift from 430 nm to 424 nm, as well as a slight shoulder forming at 411 nm. Upon the titration of compound 3.2 with DABCO, the UV spectrum also showed an H-type aggregation based on the Soret band shift from 428 to 424 nm, indicative of a blue shift. There was also a slight increase in the shoulder peak at 413 nm, which also corresponds to a blue shift.¹¹³,¹¹⁴ Titration of 3.1 with pyridine as the ligand, also showed
an H-aggregate based on the UV spectrum in the Soret band, shifting from 430 to 427 nm with a slightly lower intensity of the absorption. For compound 3.2, titration with pyridine probed by UV spectroscopy demonstrated an H-aggregate again in the Soret band, with a very slight shift from 430 to 426 nm with similar absorption intensity. Overall, these compounds react similarly to the addition of both ligands; all UV analysis demonstrated an H-type aggregation upon the titration of each ligand with each tetrapeptide (figure 3.4).
Porphyrrins in general, have a unique characteristics using circular dichroism (CD) depending on their stacking, or interactions with an external source. For example, when two or more porphyrin units are chirally oriented near each other in space, a situation called exciton coupling can occur due to the coupling of their excited states. This coupling causes the exited states to become non-degenerate; it also causes an intense
bisignate CD signal, characteristic of this stacking. The intensity is based on the distance between the chromophores that are interacting. Also, as mentioned previously, the Soret band of porphyrins has a higher intensity than the Q bands; it consists of two polarized transitions that are perpendicular to each other. Since the system in this study is an optically active porphyrin system, the CD spectrum can give insight into the distance and orientation of the porphyrins due to its high sensitivity. The addition of a linker between porphyrin units, in this case DABCO, can further fix the conformations of the Zn-TPP units, shown in the CD as a bisignate signal.

CD spectroscopy was also used to investigate the responsiveness of each peptide with the two ligands, DABCO and pyridine. TFE could be used again as the solvent for the control spectrum of each peptide for CD due to its disruption of the assembly. In TFE, the CD spectrum revealed a flat line, demonstrating a lack of observable chirality in the supramolecular assembly of the Zn-porphyrin conjugates for both compound 3.1 and 3.2. In chloroform, however, the CD spectra of 3.1 showed a slightly negative exciton cotton effect resulting in an insignificant bisignate signal, corresponding to the non-specific aggregation that is shown in TEM. Compound 3.2 showed a relatively strong exciton cotton effect in the CD spectrum in the range of 380-460 nm, containing a bisignate exciton coupling between 400 and 432 nm, as well as 432-460 nm (Figure 3.4). Both strongly positive helical exciton Cotton effects showed significant overlap, correlating to the interactions of the porphyrin chromophore and its absolute orientation, based on the exciton chirality method. When the nanostructures form, the porphyrin rings interact, resulting in a specific chiral array of P-type helical, intermolecular packing. The bisignate signal with the positive cotton effect at a longer wavelength and
the negative cotton effect at a shorter wavelength, demonstrated in compound 3.2 in chloroform, is indicative of p-type packing of the chromophores. This corresponds to the exciton coupled CD, with a strong diagnostic CD Soret signal, as well as a broad absorbance of the Soret π-π* transition of the Zn-TPP chromophore in chloroform.

The addition of each ligand caused changes in the CD spectra for both DABCO and pyridine. Upon addition of DABCO (1 eq) to compound 3.1, the CD spectrum showed a large decrease in the exciton coupling between 432 and 460 nm, and a new positive exciton coupling between 423 and 460 nm, as well as a negative exciton coupling between 390 and 423 nm (figure 3.5). The new peaks that were observed had a strong intensity and demonstrated a red shift upon addition of DABCO compared with compound 3.1 dissolved in chloroform without a ligand. Upon addition of the DABCO ligand, the CD spectroscopy confirms a change in the configuration of the Zn-TPP chromophore stacking interactions, based on the change in the exciton and electronic transitions seen in the CD spectrum.

Compound 3.2 was also combined with DABCO (1 eq) in chloroform; the CD spectrum showed a decrease in exciton coupling between 432 and 460 nm, with a new, strong peak appearing as a negative exciton coupling between 400 and 432 nm (figure 3.5). This observation indicates a more complex phenomenon other than long-range exciton coupling between Zn-TPP moieties. Based on this observation, it seems that the addition of DABCO leads to the coordination of two Zn-TPP molecules with one molecule of DABCO in between them, resulting in a new configuration of the chiral building blocks. When in this configuration, the chiral supramolecular array is overstated in the CD spectrum leading to the new exciton signal between 400 and 432
nm, with a hypsochromically shifted band appearing at 413 nm (figure 3.5). The excellent stimuli-responsiveness of this assembly could also contribute to the increase in exciton coupling in the CD, driven by the sergeant-soldier effect of an H-type coordination of two Zn-TPP units with DABCO. By coordinating DABCO, the π-π stacking would increase between neighboring Zn-TPP moieties, and it could contribute to the chiral amplification of the supramolecular assembly observed by the exciton transition on the CD. By adding the DABCO ligand, strong coordination and π-π stacking of the porphyrin units during assembly can lead to self-quenching of fluorescence.

When pyridine (1 eq) was added to both compound 3.1 and 3.2, the CD spectra showed a gradual decrease in the exciton coupling cotton effect in the region from 400 to 460 nm. Pyridine can cap the axial position of the Zn-TPP moiety, causing the decrease in signal intensity due to the prevention of amplification of the π-π intermolecular packing of the chromophores. The addition of DABCO showed a strong, broad exciton-coupling signal between 400 and 460 nm, while addition of pyridine demonstrated a decrease in intensity in the same region on the CD (figure 3.5). The negative exciton Cotton effect emerged when the peptides were dissolved in chloroform, corresponding to both of the π-π* absorption peaks of the Zn-TPP molecule. The CD also resulted in a negative chirality, representative of an M-type helical, intermolecular packing orientation of the Zn-TPP moieties embedded in the nanostructure.
Imaging the nanostructures of both compound 3.1 and 3.2 using transmission electron microscopy (TEM), atomic force microscopy (AFM), and scanning electron microscopy (SEM) methods could further validate the structural transitions shown in the UV and CD spectra. A structural transition can be observed using microscopy techniques for both tetrapeptides 3.1 and 3.2 in chloroform, with a difference in structure depending on the ligands. In TFE, no structures could be observed for both peptides or using compound 3.1 in chloroform; in chloroform, very few one-dimensional structures could be observed for compound 3.2, with a height of 6.5 nm, and a width of 31.5 nm. Addition of a nitrogen containing ligand like DABCO initiated a molecular recognition between zinc and the ligand. Therefore, a continuous, perpendicular axial Zn-TPP and amine ligand coordination can drive one-dimensional assembly of compound 3.2 into an
organogel in chloroform. Using the microscopy methods mentioned previously, an extensive network of long nanofibers was observed with a height of 5.5 nm and a width of 12.7 nm. Using pyridine as the ligand on the other hand, caps the axial position of the Zn-TPP complex, preventing elongation of the nanofibers. Therefore, short nanofibers were observed with a width of 11.5 nm and a height of 6.8 nm (figure 3.6).

Similar effects were observed using compound 3.1 in chloroform depending on the interactions of the ligands. When 3.1 was dissolved in chloroform without a ligand present, non-specific aggregations formed shown by TEM. The addition of DABCO drives formation of 1-D nanofibers by cooperative self-assembly; the fibers had a width of 14.5 nm and a height of 6.5 nm. Upon addition of pyridine with compound 3.1, liposome structures were observed using spectroscopic methods (figure 3.6); they had a uniform diameter of 251 nm. Based on the heights observed for both compound 3.1 and 3.2, both seem to be made of a bilayer type assembly process. The calculated length of one of the stacking amphiphilic molecules is 3.3 nm using molecular dynamics and conformational analysis calculations. Using AFM, compound 3.1 with the addition of pyridine showed an additional structure besides liposomes, a very fine nanofiber with a height of 1.6 nm. The minute height of this structure indicates a monolayer assembly that folds up during the aggregation process.
Figure 3.6 TEM image of compound 3.1 in chloroform with no ligand (A), with DABCO (B), or with pyridine (C); TEM image of compound 3.2 in chloroform with no ligand (D), with DABCO (E), or with pyridine (F).

In order to investigate the β-sheet self-assembly mechanism of both compound 3.1 and 3.2, one amino acid was labeled with a $^{13}$C in its peptide bond (compounds 3.3 and 3.4 respectively). In this case, the phenylalanine amino acid in the tetrapeptides was isotopically labeled at the α-carbon position. Infrared spectra have been used to study secondary structures of polypeptides using isotopically edited sequences compared to the respective non-isotope structures. The difference between the isotopically labeled peptide with its respective non-isotope is in the amide I band on the IR spectrum; in the isotope spectra, the band splits at certain wavelengths if the labeled peptide forms an anti-parallel β-sheet assembly or a parallel β-sheet assembly. Axelsen and coworkers studied
this strategy to differentiate between parallel and anti-parallel β-sheet structures (figure 3.7). The hypothesis we made was that there was a possibility of a change between parallel and anti-parallel β-sheets upon addition of the DABCO ligand. We used IR studies using isotopic equivalents of the tetrapeptides to investigate this idea.

Figure 3.7 Hydrogen bonding difference between parallel and anti-parallel β-sheets.
For the IR studies, all four tetrapeptides were dissolved in chloroform with no ligand, with DABCO (1 eq), or with pyridine (2 eq). Compound 3.1 showed a strong absorption of the amide II band at 1550 cm$^{-1}$, and an amide I absorption at 1628 cm$^{-1}$ with a weaker absorption at 1686 cm$^{-1}$. The addition of both DABCO and pyridine ligands resulted in a slight change of the amide I peak at 1630 cm$^{-1}$, as well as a weaker absorption at 1682 cm$^{-1}$. These peaks correspond to the widely accepted absorptions for β-sheets, mentioned previously. The isotopic equivalent of compound 3.1, compound 3.3, showed a split in the amide I band with peaks at 1602 and 1638 cm$^{-1}$, corresponding to an anti-parallel β-sheet assembly mechanism. The addition of DABCO also gave the split amide I band at 1600 cm$^{-1}$ and 1638 cm$^{-1}$; finally, pyridine addition resulted in a split amide I band in the IR spectrum with peaks at 1600 cm$^{-1}$ and 1638 cm$^{-1}$. Since these peaks split away from the peak of the non-isotopic tetrapeptide peak, it indicates a change in the interaction of the carbonyl stretching peaks; the $^{12}$C and $^{13}$C peaks are interacting with each other as opposed to the $^{12}$C lining up with another $^{12}$C and the $^{13}$C lining up with another $^{13}$C in parallel β-sheets. Since this is the case for all three sets of conditions, there is no change between parallel and anti-parallel β-sheets. If there was a parallel β-sheet interaction, there would be a peak similar in wavelength to the non-isotope representing the $^{12}$C-$^{12}$C interaction, as well as a peak at a lower wavelength corresponding to the $^{13}$C-$^{13}$C interaction. The amide I splitting pattern, even with the addition of each ligand, confirms the anti-parallel β-sheet formation regardless of the conditions (figure 3.8).

Compound 3.2 showed similar peak changes in the isotope compared to the original tetrapeptide on the IR spectra. When 3.2 was dissolved in chloroform without
the presence of a ligand, the IR showed a strong absorption of the amide II band at 1550 cm\(^{-1}\), and the amide I band at 1629 cm\(^{-1}\) with a weaker absorption at 1681 cm\(^{-1}\). The IR spectra after the addition of each ligand presented a minimal change in the amide I band at 1630 cm\(^{-1}\) and the weaker absorption present at 1681 cm\(^{-1}\). When the phenylalanine amino acid was isotopically labeled (compound 3.4) the IR spectra when dissolved in chloroform showed a split amide I band with peaks at 1602 cm\(^{-1}\) and 1632 cm\(^{-1}\), corresponding to the anti-parallel \(\beta\)-sheet formation. Addition of DABCO also showed evidence of anti-parallel \(\beta\)-sheets, with a split amide I band at 1604 cm\(^{-1}\) and 1634 cm\(^{-1}\) on the IR spectrum. Addition of pyridine showed a similar set of peaks; the amide I band split at 1601 cm\(^{-1}\) and 1633 cm\(^{-1}\) also corresponding to anti-parallel \(\beta\)-sheets. Even though both tetrapeptides 3.1 and 3.2 demonstrated differences in their nanostructures via microscopy methods, each retains an anti-parallel \(\beta\)-sheet assembly in each set of conditions (figure 3.8).
Figure 3.8 IR spectra for compound 3.1 in chloroform with no ligand (black), with DABCO (red), and with pyridine (blue) (A). IR spectra for compound 3.3 in chloroform with no ligand (black), with DABCO (red), and with pyridine (blue) (B). IR spectra for compound 3.2 in chloroform with no ligand (black), with DABCO (red), and with pyridine (blue) (C). IR spectra for compound 3.4 in chloroform with no ligand (black), with DABCO (red), and with pyridine (blue) (D).

Finally, an experiment was performed to demonstrate the recyclability of DABCO in the assembly mechanism. To compound 3.2 (100 µM) in chloroform, one equivalent of DABCO and one equivalent of TFA were added sequentially, with a CD taken after each equivalent was added. DABCO increases the pH to be slightly basic at 8.9, corresponding to an increase in the exciton coupling cotton effect in the range of 390 to
460 nm. The increase in exciton coupling indicates an increase in chiral π-π stacking between the Zn-TPP chromophore from the axial Zn-TPP coordination with the DABCO ligand. When TFA is added to the system, the pH becomes acidic at 3.5, completely flat-lining the signal due to protonation of DABCO, thus eliminating the coordination with the Zn-TPP moiety. This cycle could be repeated several times, indicating a disruption and reformation of the assembled system.

3.4 Conclusion

Overall, the two tetrapeptides 3.1 and 3.2 demonstrate stimuli-responsiveness to its chemical and physical environment in chloroform. The Zn-TPP assemblies interact differently depending on the conditions and were monitored by various spectroscopic and microscopic methods to determine their interactions. The isotopically labeled tetrapeptides were also synthesized to determine if the assembly mechanism was forming parallel or anti-parallel β-sheets. Based on the IR spectra, the structures that were predominantly formed were anti-parallel β-sheets, regardless of the conditions. The versatile morphology of these tetrapeptides can be used for future biomedical and materials development.

3.5 Methods and Experimental

General Methods

Fourier transform-infrared (FTIR) spectroscopy was performed using a Shimadzu IRAffinity-1S, FTIR spectrometer. Circular dichroic (CD) spectra were taken with a JASCO CD spectrometer. Atomic force microscopy (AFM) was conducted in tapping mode. All fluorescence spectroscopy were performed on a Shimadzu RF-6000
instrument using a cuvette with 1 mm or 1 cm pass length at 25°C. ESI or FAB mass spectra were recorded at the Bruker MicrOTOF MS instrument in The Ohio-State University Chemical Instrument Center-Chemistry. Transmission electron microscopy (TEM) was performed with a Technai G2 Spirit instrument operating at 80 kV. All reactions were performed under an argon or nitrogen atmosphere. $^1$H NMR were recorded at 400 or 500 MHz and $^{13}$C NMR spectra at 100 or 125 MHz on a Bruker DPX-400 or DPX-500 instrument as indicated. Dimethylformamide (DMF) was dried by distillation from MgSO4. Chromatographic separations were performed on silica gel 60 (230-400 mesh, 60 Å) using the indicated solvents. All chloroform used for sample solutions was HPLC grade and passed through membrane filter (0.02 µm). Fmoc-lys-OH, Fmoc-Phe-OH and Fmoc-lys(Boc)-OH was purchased from Novabiochem and used without further purification. Chromatographic separations were performed on silica gel 60 (230-400 mesh, 60 Å) using the indicated solvents. Peptide purity was assessed by analytical reverse-phase HPLC and identity confirmed using ESI mass spectrometry.
**Synthesis of Fmoc-Lys(TPP)-OH**

![Chemical reaction diagram]

**TPP-COOMe 3.1**: Pyrrole (3.6 mL, 55.5 mmol), benzaldehyde (3.1 mL, 28.1 mmol), and methyl-4-formyl benzoate (1.64 g, 9.99 mmol) were dissolved in dichloromethane (2 L). The reaction vessel was covered in foil, and degassed using argon gas. BF$_3$O(Et)$_2$ (0.433 mL) was added to the solution, and the resulting mixture was allowed to stir for 16 h. The reaction was quenched using chloranil (6.13 g) for 3 h. The solvent was removed, and the resulting brown solid was purified via flash column chromatography (2:1 hexanes: DCM, to 1:1 hexanes: DCM, to pure DCM) to yield a purple solid (1.0956 g, 16%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.86-8.88 (m, 6H), $\delta$ 8.81 (d, 2H), $\delta$ 8.44-8.46 (d, 2H), $\delta$ 8.31-8.33 (d, 2H), $\delta$ 8.21-8.24 (d, 6H), $\delta$ 7.73-7.81 (m, 9H), $\delta$ 4.12 (s, 3H).
TPP-COOH 3.2: Compound 3.1 (1.096 g) was dissolved in THF (80 mL). NaOH (10%, 54 mL) was added, and the resulting mixture was heated to 80°C and stirred for 16 h. Reaction cooled to room temperature, and concentrated hydrochloric acid was added until the solution turned from purple to green. The solution was then washed with chloroform, then water. The organic layers were combined and dried using sodium sulfate. The solvent was removed, and the remaining solid was redissolved in dichloromethane for purification via flash column chromatography (1:1 DCM: hexanes, DCM, 1% MeOH-5% MeOH in DCM) to yield a purple solid (0.7565 g, 68%). $^1$H NMR $\delta$ 8.86-8.89 (m, 6H), 8.81 (d, 2H), 8.50-8.52 (d, 2H), 8.21-8.24 (d, 6H), 7.76-7.80 (m, 9H).
**Fmoc-Lys(TPP)-OMe 3.3**: Compound 3.2 (0.398 g, 0.604 mmol) was dissolved in dichloromethane. HOBr (0.0952 g, 0.604 mmol), HBTU (0.2278 g, 0.604 mmol), Fmoc-Lys-OMe (0.02367 g, 0.604 mmol), and DMAP (0.0078 g, 0.0604 mmol) were added to the reaction mixture. The reaction stirred for 12 h, and the remaining solution was washed with water. The organic layer was collected and dried over Na$_2$SO$_4$. DCM was removed in vacuo, and the purple oil was purified via column chromatography (2% MeOH in CHCl$_3$) to yield a purple solid (0.248 g, 41%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.71-8.75 (m, 5H), 8.61 (d, 2H), 8.06-8.61 (m, 7H), 8.00 (d, 2H), 7.61-7.66 (m, 9H), 7.36-7.46 (m, 5H), 7.02-7.19 (m, 5H), 6.589 (b, 1H), 5.548 (d, 1H), 4.32-4.34 (m, 1H), 4.26-4.32 (m, 1H), 4.07 (q, 1H), 3.89-3.92 (m, 1H), 3.72-3.80 (m, 1H), 3.69 (s, 4H), 3.48 (d, 1H), 1.82-1.96 (m, 1H), 1.22-1.26 (m, 1H), 1.13 (d, 2H). ESI-MS calculated for C$_{67}$H$_{54}$N$_6$O$_5$ (M+H$^+$) = 1023.4189, found 1023.3227.
**Fmoc-Lys(TPP)-OH 3.4:** Compound 3.3 (2.24 g, 2.18 mmol) was dissolved in 1,2-dichloroethane (20 mL). Trimethyltin hydroxide (made by reacting trimethyltin chloride with potassium hydroxide, 3.95 g, 21.8 mmol) was added to the mixture and the resulting solution was heated to 70°C for 12 h. The solvent was removed in vacuo, and the residue was redissolved in dichloromethane. The organic layer was washed with aqueous KHSO₄ (0.01 M, 30 mL) and brine (30 mL), and dried over sodium sulfate. The solvent was removed in vacuo, resulting in the crude product, which was purified by flash column chromatography (2% MeOH to 5% MeOH in CHCl₃) to yield Fmoc-Lys(TPP)-OH (670 mg, 66%) as a purple powder. \(^1\)H NMR (400 MHz, CDCl₃) 1.123 (d, 2H), 1.215-1.243 (m, 1H), 1.833-1.963 (m, 1H) 3.423 (d, 1H), 3.612-3.693 (m, 1H), 3.719-3.801 (m, 1H), 3.892-3.923 (m, 1H), 4.075 (q, 1H) 4.259-4.318 (m, 1H), 4.318-4.344 (m, 1H), 5.548 (d, 1H) 6.589 (b, 1H), 7.019-7.194 (m, 5H), 7.361-7.459 (m, 5H), 7.611-7.663 (m, 9H), 8.002 (d, 2H), 8.056-8.606 (m, 7H), 8.613 (d, 2H), 8.713-8.754 (m, 5H). ESI-MS calculated for C₆₆H₅₂N₆O₅ (M+H⁺) 1009.4033, found 1009.3516.
Peptide preparation
All peptide-TPP conjugates were manually prepared using Fmoc solid-phase synthesis on the rink amide resin (loading 0.8 mmol/g). Amide-coupling steps were performed with standard techniques for each amino acid: Fmoc-amino acid, 1,3-diisopropylcarbodiimide (DIC), 1-hydroxybenzotriazole (HOBt), and 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (200 mol% relative to each resin) in 1:1 DCM:DMF for 3 h, except for the TPP-modified amino acid (12-18 h). Piperdine (20%) in DMF was used to remove all Fmoc groups (3x30 min). The peptide-TPP hybrids were cleaved from the resin using 95:2.5:2.5 TFA/TES/water by shaking for 2 h at room temperature. The peptides were precipitated into diethyl ether, and redissolved in 1:1 water/acetonitrile for purification via reverse phase HPLC. The peptides were confirmed using ESI-MS.
Synthesis of Zinc-TPP conjugates

Fmoc-FKK(Zn-TPP)K-NH₂ (A and B): All peptide-TPP conjugates were manually prepared by adding zinc acetate to a stirring mixture of peptide-TPP conjugates in 10% CH₃CN/water. The resulting mixture was stored as lyophilized powers at 0 °C. The resulting crude solids were precipitated and wash with water (3 x 30 mL) before purification by reversed-phased HPLC on preparative Water C18 column eluting with a linear gradient of CH₃CN/water (30/70 to 100/0 over 40 minutes, 0.1 % TFA) and stored as lyophilized powers at 0 °C. Peptide purity was assessed by analytical reverse-phase HPLC and identity confirmed using ESI-TOF mass spectrometry.

3.6 References


Bibliography


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92


Appendix A: NMR data
Appendix B: HPLC Traces and Mass Spectrums

Fmoc-FKK(TPP)K-NH₂
Fmoc-FKK(TPP-Zn)K-NH₂

[M+H]⁺=1497.5404
Fmoc-$^{13}$FKK(TPP)K-NH$_2$

\[
[M+H]^+ = 1413.6555
\]
Fmoc\textsuperscript{13}FKK(TPP-Zn)K-NH\textsubscript{2}

\[ [M+H]^+ = 1498.5453 \]
Fmoc-FKacK(TPP)K-NH$_2$

$[\text{M+H}]^+ = 1454.6601$
Fmoc-$^{13}$FKacK(PP)K-NH$_2$

$[M+H]^+=1455.6584$
Fmoc-FKacK(PP-Zn)K-NH$_2$

$[M+Na]^+=1538.5513$
Fmoc-^{13}FKacK(PP-Zn)-NH\textsubscript{2}

\[ \text{[M+Na]}^+ = 1540.5537 \]